

DNA Mismatch Repair Gene Mutations in Human Cancer

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A new pathogenetic mechanism leading to cancer has been delineated in the past 3 years when human homologues of DNA mismatch repair (MMR) genes have been identified and shown to be involved in various types of cancer. Germline mutations of MMR genes cause susceptibility to a hereditary form of colon cancer, hereditary nonpolyposis colon cancer (HNPCC), which represents one of the most common syndromes associated with cancer predisposition in man. Tumors from HNPCC patients are hypermutable and show length variation at short tandem repeat sequences, a phenomenon referred to as microsatellite instability or replication errors. A similar abnormality is found in a proportion of sporadic tumors of the colorectum as well as a variety of other organs; acquired mutations in MMR genes or other endogenous or exogenous causes may underlie these cases. Genetic and biochemical characterization of the functions of normal and mutated MMR genes elucidates mechanisms of cancer development and provides tools for diagnostic applications. — *Environ Health Perspect* 105(Suppl 4):775–780 (1997)

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Discovery of Human Mismatch Repair Genes

The existence of mismatch repair (MMR) enzymes in bacteria has been known for at least two decades and more recently, similar activities have been identified in yeast and higher eukaryotes (1). The discovery of human MMR genes was greatly facilitated by advances made in genetic studies of hereditary nonpolyposis colorectal cancer (HNPCC). After the first HNPCC susceptibility locus was mapped to chromosome 2p by linkage analysis (2), it was found that tumors from HNPCC patients showed instability at multiple random microsatellite sequences throughout the genome (3). A similar phenotype had previously been

observed in bacterial and yeast strains with DNA mismatch repair gene mutations (4,5). These data together provided a functional clue that resulted in the identification of human homologues of bacterial and yeast MMR genes (Figure 1).

Functions of the MMR System

In *Escherichia coli*, mutS and mutL proteins participate in two main repair pathways, the methyl-directed long-patch and the very short-patch (VSP) pathway (1,16). The methyl-directed pathway functions by correcting base–base mispairs, small insertions and deletions resulting from errors in DNA replication. The specific function of the VSP pathway is to correct G–T mispairs in nonreplicating DNA that arise as a consequence of deamination of 5-methylcytosine residues. Methyl-directed mismatch repair in *E. coli* depends on 10 activities. Repair is initiated by binding of mutS to the mismatch, followed by the addition of mutL. This complex activates mutH, an endonuclease, which makes a nick at a GATC site with unmethylated adenine, located 1 to 2 kb from either side of the mismatch. Subsequently, the portion containing the mismatch is excised by a 3′–5′ or 5′–3′ exonuclease and replaced by a new tract synthesized by a DNA polymerase. In yeast, after recognition of the mismatch by MSH2, a heterodimer is formed by MLH1 and PMS1, followed by a ternary complex

formation by MLH1, PMS1, and MSH2 (17). This complex then recruits additional proteins that accomplish the repair as in the bacterial system.

The human MMR system is believed to operate in more or less the same general fashion. Biochemical analyses in human cells have demonstrated that repair is strand-specific and is directed by a nick located 5′ or 3′ to the mismatch (18,19). The mismatch-binding factor in humans consists of two distinct proteins, the 100-kD MSH2 and a recently identified 160-kD polypeptide, G/T mismatch binding protein (GTBP) (14). While *E. coli* has a single *mutL* gene, human cells have at least 16 genes that specify mutL-like proteins (Figure 1); whether they have different functions with respect to each other is not known. Recent studies have revealed some differences between MMR proteins in their capacities to repair specific types of biochemical defects. While MSH2 and MLH1 seem to be equally important in single base mismatch repair, MSH2 plays a more prominent role in the repair of loops containing five or more unpaired bases (20). Human cells may be capable of repairing loops of up to 14 nucleotides (21), which is important since human DNA contains numerous microsatellites that may generate large loops as a consequence of strand slippage during replication. Furthermore, it is suggested that GTBP is necessary for the correction of base–base mispairs and one- or two-nucleotide loops but may not be absolutely required in the correction of larger loops (22,23) (see also Figure 2).

Apart from being responsible for the correction of biosynthetic errors in newly synthesized DNA, the MMR system is also presumed to repair exogenous chemical damage, such as lesions caused by alkylating agents (16). Furthermore, the system is implicated in the prevention of recombination between quasi-homologous (homeologous) sequences that have diverged genetically (24).

Microsatellite Instability and Its Occurrence in Cancer

Instability at short tandem repeat sequences (microsatellites) reflects malfunction in the replication or repair of DNA. For this reason, it is also referred to as the replication error (RER) phenomenon (3). Biochemical studies by Parsons et al. (18) provided a link between microsatellite instability and defective mismatch repair. The abnormality

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Abbreviations used: ERCC2, excision repair cross complementing 2; GTBP, G/T mismatch binding protein; HNPCC, hereditary nonpolyposis colon cancer; MLH1, mutL homolog 1; MMR, DNA mismatch repair; MSH2, mutS homolog 2; PMS1, postmeiotic segregation increased 1; PMS2, postmeiotic segregation increased 2; RER, replication error; VSP, very short-patch pathway.

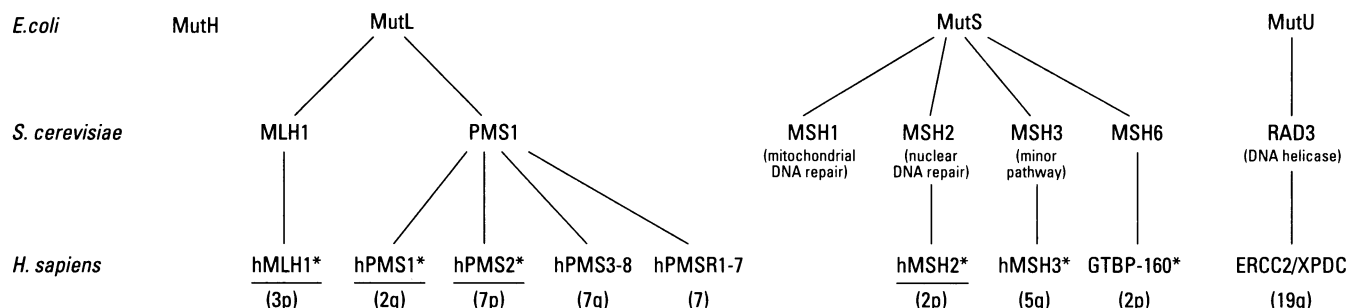


Figure 1. Mut family of DNA mismatch repair genes. Location on human chromosomes is given in parentheses. Genes whose germline mutations cause predisposition to HNPCC are underlined and those shown to be associated with microsatellite instability in human cancer are indicated with an asterisk. The references for human sequences are as follows: hMLH1 (6,7); hPMS1 and hPMS2 (8); hPMS3-8 (9); hPMSR1-7 (10); hMSH2 (11,12); hMSH3 (13); GTBP-160 (14); and ERCC2/XPDC (15).

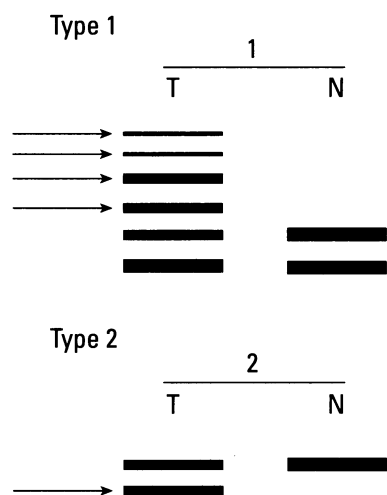


Figure 2. Schematic illustration of two main patterns of microsatellite instability, as seen after gel electrophoresis of PCR-amplified microsatellite sequences. N, normal DNA; T, tumor DNA. Aberrant fragments are indicated by arrows. Patient 1 is constitutionally heterozygous and patient 2 homozygous for the studied microsatellite marker. Type 1 consists of a ladder of extra fragments; a majority (30–70%) of microsatellite loci that have been altered; and mono-, di-, tri-, or tetranucleotide repeats that are involved. It is caused by mutations in HNPCC-related MMR genes and is characteristic of HNPCC tumors and sporadic tumors, which are typically part of the HNPCC tumor spectrum (31). Type 2 consists of a single extra fragment, a minority (a few percent) of microsatellite loci that have been altered, and mononucleotide repeats (23) or tri- and tetranucleotide repeats (25) that are primarily involved. It is caused by mutations in DNA polymerase δ (26), GTBP-160 (23), *MSH3* (27); or possibly other MMR genes, or exposure to chemical carcinogens (28). It is characteristic of sporadic tumors that may or may not be part of the HNPCC spectrum.

appears as extra alleles observed in tumor DNA when compared to normal DNA from the same individual (Figure 2). These aberrant-sized alleles result from a gain or loss of short repeat units [e.g., CA dinucleotides in a (CA) n repeat] that may

be caused by DNA polymerase slippage (combined with inability to repair these kinds of defects).

Microsatellite instability serves as a useful marker of a “mutator” phenotype characteristic of HNPCC and some sporadic tumors. Approximately 100,000 microsatellite repeats are scattered throughout the human genome (29). About 90% of colorectal cancers from HNPCC patients show microsatellite instability and a majority of microsatellite loci are apparently involved (3,30). Thus, in HNPCC tumors, the total number of mutations at microsatellite loci alone could be as high as 100,000 per cell. As shown in Table 1, approximately 15% of apparently sporadic colorectal carcinomas and a variable proportion of other cancers also show this abnormality. The genetic background of microsatellite instability may not be the same in HNPCC and various sporadic cases. In HNPCC, the phenotype results from inactivation of one of four MMR genes, namely, *MSH2*, *MLH1*, *PMS1*, or *PMS2*. While structural defects in these genes are demonstrable in a proportion of sporadic tumors as well (55), a significant fraction may arise by other mechanisms, including mutations in other genes, for example DNA polymerase δ (26). Even exogenous damage has been implicated (28). The pattern of microsatellite instability may vary according to etiology (Figure 2).

Model of Carcinogenesis in HNPCC and Sporadic Tumors with MMR Deficiency

Molecular genetic studies support the idea that multiple genetic changes are necessary for tumorigenesis. Statistically, it has been estimated that colorectal tumors require four to seven mutations to develop (56). Colorectal carcinoma cell lines with MMR

deficiency are hypermutable with mutation rates that can be several hundredfold compared to normal human cells (18,57). Importantly, mutations in HNPCC-related MMR genes cause significantly elevated mutation rates not only in repetitive sequences but in nonrepetitive gene sequences (such as the locus for hypoxanthine guanine phosphoribosyltransferase) as well (57).

Figure 3 shows a model of colon cancer development in HNPCC. The basic principles of this model are applicable to any tumors with inactivation of both copies of a MMR gene as an early event in their pathogenesis. In tumors with MMR deficiency, a classical tumor suppressor mechanism is supported in that two hits are required to cause a phenotypic effect (18,55,58). Most MMR gene mutations are point mutations resulting in truncated protein products (59,60). Loss of a critical MMR activity as a consequence of these mutations is presumed to occur early in tumor development, already at the adenoma stage (61). Ensuing genetic destabilization initiates a cascade of further mutations (62), probably targeting at different genes in tumors from different organs. In colon cancer, multiple somatic mutations, primarily single nucleotide deletions and G:C to A:T transitions, have been reported to occur in *APC* and *p53* genes (63). Furthermore, inactivating frameshift mutations in a polyadenine tract located in the coding sequence of the transforming growth factor β receptor II (TGF β -RII) gene are frequent in colon, but rare in endometrial cancers with microsatellite instability, which suggests different pathogenetic routes in these tumors (64,65).

In some instances, even one hit (constitutional heterozygosity for an MMR gene mutation) may be sufficient for MMR deficiency to become manifested (66).

Table 1. Frequency of microsatellite instability (percent of tumors) in sporadic cancers. HNPCC spectrum refers to tumors that occur in excess in HNPCC kindreds compared to the general population (31).

Organ	Part of HNPCC spectrum	Frequency, %	Note	Reference		
Colorectum	Yes	12	Poly(A) sequences studied	(32)		
		28 ^{a,b}		(33)		
		16 ^a		(30)		
		16.5 ^{a,b}		(34)		
Endometrium	Yes	17 ^a		(35)		
		22 ^a		(36)		
		20		(37)		
Stomach	Yes	39		(38)		
		31 ^a		(39)		
		32 ^a		(40)		
Pancreas	Yes (?)	67	Epithelial cancer	(38)		
Ovary	Yes	0		(41)		
		16		(38)		
		10 ^b		(25)		
Kidney (adenocarcinoma)	Yes	25 ^{a,b}			(42)	
Breast	No	0		(36)		
		4		(38)		
		20 ^a		(43)		
		11 ^b		(25)		
Lung cancer	No	45 ^a		(44)		
Small cell						
Non-small cell					2 ^b	(36)
					34 ^{a,b}	Loci on 3p mainly involved
		6.5	(46)			
		21 ^b	Loci on 3p mainly studied	(47)		
Brain	No	2 ^b		(25)		
Testis (germ cell)	No	0		(36)		
Bladder	No (?)	18 ^{a,b}	Loci on 1q involved	(48)		
		3 ^{a,b}		(49)		
		41 ^b		Chromosome 9-specific loci studied	(50)	
		28	Tri- and tetranucleotide repeats studied	(51)		
Head and neck	No	29	Tri- and tetranucleotide repeats studied	(51)		
Esophagus	No	22 ^b		(52)		
Adenocarcinoma						
Squamous cell carcinoma	No	2		(52)		
Prostate	No	65 ^a		(53)		
Chronic myeloid leukemia	No	0		(54)		
Chronic phase						
Blast crisis	No	53 ^a		(54)		

^aMicrosatellite instability at ≥ 2 loci (Type 1 in Figure 2). ^bMicrosatellite instability at 1 locus only (Type 2 in Figure 2).

Despite elevated mutation rates in all tissues examined, these patients had surprisingly few tumors. It was hypothesized that mutations per se might not be sufficient for a high rate of tumorigenesis, but that other factors would be necessary, such as exposure to exogenous mutagenic compounds that not only induce mutations but also stimulate the cells to regenerate (66).

Recently, a time (rather than replication) dependent model involving the MMR system was proposed to generate multiple mutations in cancer. In this model (67) it is assumed that whenever MMR mechanisms act on mismatched bases in nondividing

cells, they will be unable to distinguish the nascent and old strands and therefore [analogous to nucleotide excision repair mechanisms (68)] can lead to mutation fixation rather than elimination. This could explain the excessive numbers of new mutations found in tumor cells of otherwise normal (i.e., non-HNPCC) individuals and the increasing cancer incidences according to age (67).

Future Prospects

Now that at least a proportion of human MMR genes have been cloned and characterized, the possible involvement of

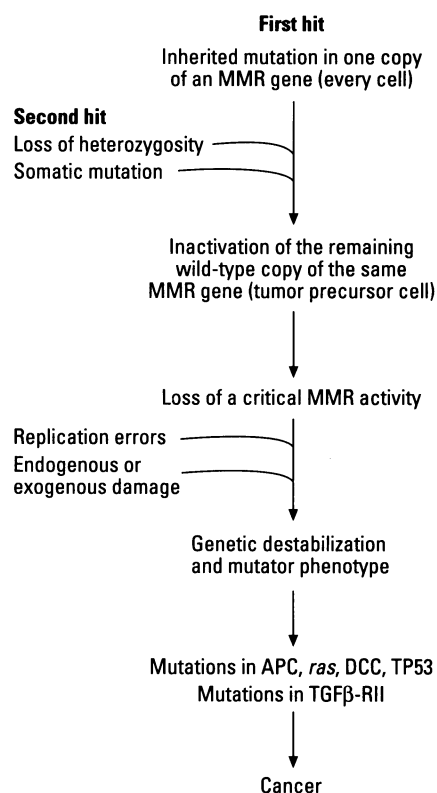


Figure 3. Model of cancer development in HNPCC.

this system in cancer can be studied using different approaches, some of which are described below:

Microsatellite Instability in Tumor Tissue. *a*) Microsatellite sequences from normal and tumor tissue are amplified by the polymerase chain reaction and the products separated by polyacrylamide gel electrophoresis (2). *b*) The technique can be automated by the use of fluorescent primers and a DNA sequencer (69).

Biochemical Assays of MMR Capacity. *a*) Heteroduplex DNA containing single base mismatches or displaced loops is incubated with tissue extract under investigation; heteroduplex repair measured in various ways (18,70). *b*) Studies of complementation of repair by pairwise mixing of different defective extracts (19).

Direct Analyses of MMR Gene Mutations. DNA or RNA from normal tissue (while searching for germline mutations) or tumor tissue (while searching for germline or somatic mutations) is studied by different techniques (which are in part alternative, in part complementary), such as reverse transcriptase-PCR, protein truncation test, single-strand conformation

polymorphism analysis, denaturing gradient gel electrophoresis, two-dimensional DNA electrophoresis, and sequencing (55,71–73). A problem is that there are no clear-cut mutation hot spots in MMR genes.

Functional Analyses to Study the Effects of MMR Genes and Their Mutations. *a)* Human MMR gene introduced into MMR-deficient recipient cells [from the yeast (74) from the hamster (75)], possible restoration of MMR activity evaluated by microsatellite instability or biochemical analyses (at experimental stage). *b)* Mouse models: *PMS2*, *MSH2*, and *MLH1* knock-out mice have been created that allow studies on mechanisms of

tumorigenesis and screening carcinogenic and anticancer agents (24,76–79).

Clinically, the molecular characterization of MMR gene mutations in affected individuals allows genotype–phenotype correlations, and in at-risk individuals from HNPCC kindreds, it enables predictive testing for cancer susceptibility, enhanced clinical surveillance with the aim of early cancer detection and cure, and preventive measures. From the scientific point of view, a major question that needs to be addressed by future studies is why MMR deficiency seems to play an important role in the genesis of some tumors (e.g., those overrepresented in HNPCC) but (at least based on

the absence of microsatellite instability) does not appear to be involved in others. Explanations offered so far, which need further evaluation, include *a)* structural differences in critical cancer-associated “downstream” genes (Figure 3), which may make some of them particularly susceptible to replication errors (for example, due to the presence of a repeat sequence within a coding region as in TGF β -RII, above), *b)* redundancy among members of the Mut family (80), *c)* tissue-specific differences in MMR gene expression (16), and *d)* variable exposure to exogenous carcinogens, such as alkylating agents, which may confer selective advantage to MMR-deficient cells (81).

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